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Bradykinin activates tyrosine hydroxylase in rat pheochromocytoma PC-12 cells

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Tyrosine hydroxylase is activated in PC-12 cells by bradykinin in a concentration-dependent manner with maximal stimulation occurring at 1 μM. This stimulatory effect occurs within 15 s and is maximal at 5 min. This stimulation is due to an increase in the affinity of tyrosine hydroxylase for its pterin cofactor, and can be blocked by a specific bradykinin receptor antagonist. These data indicate that bradykinin can regulate the activity of tyrosine hydroxylase in PC-12 cells.

Tyrosine hydroxylase; Bradykinin; (PC-12 cell)

1. INTRODUCTION

Tyrosine hydroxylase is the rate-limiting enzyme in the pathway concerned with the biosynthesis of catecholamines in both the central and peripheral nervous systems [1,2]. Numerous studies have shown that the activity of tyrosine hydroxylase can be increased in rat pheochromocytoma PC-12 cells in situ by depolarization with high potassium or treatments with dibutyryl cyclic AMP, calcium ionophore and phorbol esters [3-6]. Recently, Calker and Heuman [7] reported that treatment of PC-12 cells with bradykinin, a putative central nervous system neurotransmitter [8,9], increased the accumulation of inositol- 1,4,5-trisphosphate (IP_3), suggesting that PC-12 cells contain receptors that respond to bradykinin. Here, we have analyzed the effects of bradykinin on tyrosine hydroxylase activity in PC-12 cells.

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2. MATERIALS AND METHODS

2.1. *Cell culture*

The PC-12 cells were subcultured on 35-mm polylysine-treated dishes at a density of 1×10^6 cells/dish in RPMI 1640 medium supplemented with 10% heat-inactivated horse serum, 5% fetal bovine serum, streptomycin (50 μg/ml) and penicillin (50 U/ml). After 3 days in culture, cells were washed twice with oxygenated pre-warmed (37°C) Krebs-Ringer-Hepes buffer (KRH buffer), pH 7.4, containing 125 mM NaCl, 4.8 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM Hepes and 5.6 mM glucose. The cells were preincubated with KRH buffer at 37°C for 15 min prior to treatment with bradykinin.

2.2. *Assay of tyrosine hydroxylase activity*

After incubation, KRH buffer was removed and cells were immediately frozen on dry ice and scraped into a solution containing 30 mM potassium phosphate buffer (pH 6.8), 50 mM NaF and 1 mM EDTA. The suspension was homogenized, the homogenate being centrifuged at $20000 \times g$ for 10 min. The supernatant was collected and subjected to gel filtration on a Sephadex G-50 column equilibrated with 30 mM potassium phosphate buffer (pH 6.8), 10 mM NaF and 0.1 mM EDTA. Tyrosine hydroxylase activity was determined by a modification of the coupled decarboxylase assay [10]. The standard incubation medium consisted of 100 mM potassium phosphate buffer (pH 6.8), 5 mM ascorbic acid, 6500 U catalase, 5 mM EDTA, 0.2 mM L-2-amino-4-hydroxy-6-methyl-5,6,7,8,-tetrahydropteridine-HCl (6-MePtH$_4$, Calbiochem-Behring), 0.1 mM (0.1 μCi) L-[1-^{14}C]tyrosine and 65 μl supernatant enzyme (final

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volume, 100 μl). The reaction was terminated following a 10 min incubation at 30°C by addition of 5 mM 3-iodotyrosine in 50 mM potassium phosphate buffer (pH 6.8). The [1-^{14}C]dihydroxyphenylalanine produced in the above reaction was decarboxylated by further incubation of the assay tubes for 30 min at 37°C following addition of partially purified hog kidney L-aromatic amino acid decarboxylase and pyridoxal phosphate. The reaction was then terminated by addition of 0.1 ml of 0.8 M perchloric acid. The $^{14}CO_2$ liberated was collected in wells containing 0.2 ml NCS tissue solubilizer (Amersham), and the wells were transferred to counting vials. Radioactivity was determined by liquid scintillation spectrometry. Protein content was measured by the method of Bradford [11] with bovine serum albumin as the standard. Tyrosine hydroxylase activity was expressed as nmol $^{14}CO_2$ formed/min per mg protein.

3. RESULTS AND DISCUSSION

The time course for the activation of tyrosine hydroxylase by bradykinin is shown in fig.1. Tyrosine hydroxylase activity was significantly increased in PC-12 cells following incubation with 10^{-6} M bradykinin for 15 s. Maximal activation of tyrosine hydroxylase occurred after a 5 min incubation with bradykinin (460%), and the enzyme remained activated at 10 min (380%).

Bradykinin (10^{-8} M) significantly increased tyrosine hydroxylase activity in PC-12 cells (fig.2). Maximal activation of the enzyme was achieved at 10^{-6} M bradykinin. The median effective concentration (EC_{50}) of bradykinin for the activation of tyrosine hydroxylase was approx. 3×10^{-7} M. This concentration of bradykinin is similar to that necessary to produce an approx. 400% increase in formation of inositol phosphates in PC-12 cells [7].

Fig.3 illustrates the effects of the selective

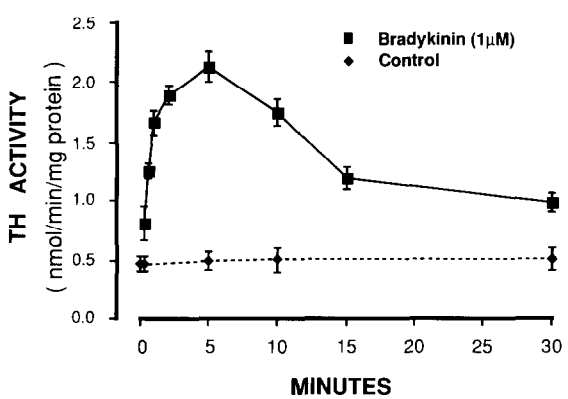

Fig.1. Time course of the effects of bradykinin on tyrosine hydroxylase activity in PC-12 cells. PC-12 cells were incubated at 37°C for different time intervals in the presence or absence of 10^{-6} M bradykinin. Tyrosine hydroxylase activity was determined as described in section 2. Data are means ± SE from 5-7 experiments. Bradykinin significantly increased tyrosine hydroxylase activity greater than control values at all time points.

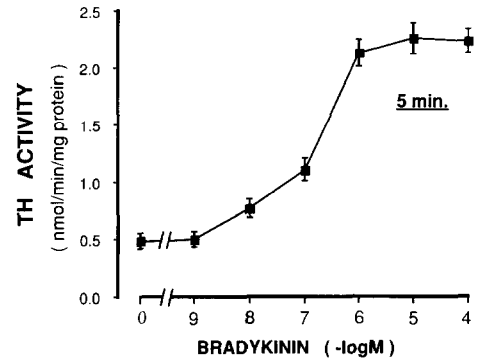

Fig.2. Effects of different concentrations of bradykinin on tyrosine hydroxylase activity. PC-12 cells were incubated for 5 min in the presence or absence of varying concentrations of bradykinin. Tyrosine hydroxylase activity was determined as described in section 2. Data are means ± SE from 5-7 experiments. Concentrations of bradykinin between 10^{-8} and 10^{-4} M are significantly greater than the 0 concentration.

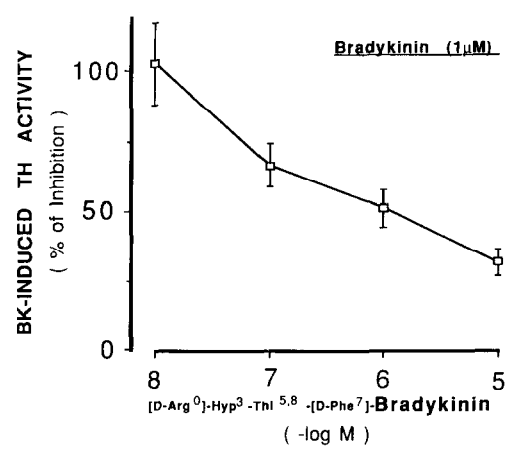

Fig.3. Inhibition of bradykinin-induced activation of tyrosine hydroxylase by bradykinin antagonist. The cells were incubated for 5 min in the presence or absence of bradykinin. The bradykinin antagonist, [D-Arg^0]-Hyp^3-Thi^{5,8}-[D-Phe^7]-bradykinin, was added 5 min before bradykinin addition. Tyrosine hydroxylase activity was determined as described in section 2. Data are means ± SE from 5-7 experiments. Concentrations of [D-Arg^0]-Hyp^3-Thi^{5,8}-[D-Phe^7]-bradykinin between 10^{-7} and 10^{-5} M significantly inhibit bradykinin-induced activation of tyrosine hydroxylase.

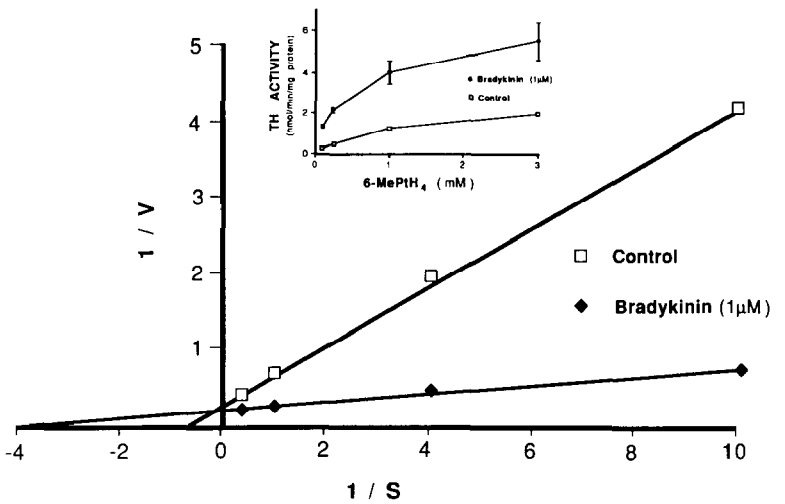

Fig.4. Kinetic analysis of the effect of bradykinin on tyrosine hydroxylase activity at various cofactor concentrations. The cells were incubated for 5 min in the presence or absence of 10^{-6} M bradykinin. Tyrosine hydroxylase activity was determined as described in section 2. All values were determined at 0.1 mM L-[1-^{14}C]tyrosine. Data are means ± SE from 3-5 experiments.

bradykinin antagonist, [D-Arg^0]-Hyp^3-Thi^{5,8}-[D-Phe^7]-bradykinin (a gift from R.J. Vavrek and J.M. Stewart, University of Colorado Health Sciences Center) on bradykinin-stimulated tyrosine hydroxylase activity. Bradykinin-stimulated tyrosine hydroxylase activity was inhibited at all concentrations of the antagonist tested with maximal inhibition occurring at 10^{-5} M (68%).

Fig.4 illustrates the kinetic analysis of the activation of tyrosine hydroxylase by 10^{-6} M bradykinin. Bradykinin produced a significant decrease in the K_m of tyrosine hydroxylase for the pterin cofactor with no change in V_{max}. The kinetic values were K_m 1.56 ± 0.21 mM and V_{max} 3.7 ± 0.4 nmol/min per mg protein for control and K_m 0.26 ± 0.04 mM and V_{max} 4.4 ± 0.6 nmol/min per mg protein for bradykinin.

Bradykinin may activate tyrosine hydroxylase by two potential mechanisms in PC-12 cells. Incubation of PC-12 cells with bradykinin produces an increase in IP_3 and presumably in diacylglycerol [7]. IP_3 has been shown to release Ca^{2+} from intracellular stores in many tissues [12]. Increasing Ca^{2+} intracellularly in the PC-12 cells may stimulate tyrosine hydroxylase by activating calcium/calmodulin-dependent protein kinase, which in the presence of an activator protein is known to activate and phosphorylate tyrosine hydroxylase [13]. In addition, diacylglycerol has also been shown to stimulate protein kinase C activity [14], which has also been shown to activate and phosphorylate tyrosine hydroxylase [6,15-17]. Therefore, the activation of tyrosine hydroxylase by bradykinin may be the result of an increase in activity of calcium/calmodulin-dependent protein kinase and/or protein kinase C leading to a direct phosphorylation and activation of tyrosine hydroxylase. We are presently evaluating the effects of bradykinin on the phosphorylation of tyrosine hydroxylase in PC-12 cells.

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